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Expression of CaBP transcripts in retinal bipolar cells of developing and adult zebrafish

Glasauer, Stella ; Neuhauss, Stephan

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Expression of CaBP transcripts in retinal bipolar cells of developing and adult zebrafish

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Abstract
Ca^{2+}-binding proteins play important roles in neuronal function by transducing Ca^{2+} signals and thereby regulating crucial processes like synaptic signaling and neuronal development, growth and survival. The Ca^{2+}-binding protein (CaBP) subfamily is part of the vast EF-hand containing calmodulin superfamily. Eight genes encoding CaBPs have been identified in zebrafish, and many of them are expressed in specific subpopulations of retinal neurons during development. Among them, *cabp2a* and *cabp5b* have been shown to be expressed in the retinal inner nuclear layer (INL). Here, we demonstrate that their paralogues, *cabp2b* and *cabp5a*, are also specifically expressed in the INL of the developing retina. By extending expression analysis of *cabp2a, cabp2b, cabp5a* and *cabp5b* to the adult retina, we reveal exclusive expression of all four genes in the INL after retinal development is completed. Thus, our findings suggest functions of *cabp2a, cabp2b, cabp5a* and *cabp5b* in Ca^{2+} signaling in mature retinal neurons, besides a role in the developing retina.

Objective
Here, we evaluate expression of *cabp5a* in the developing zebrafish retina and extend expression analysis of *cabp2a, cabp2b, cabp5a* and *cabp5b* to the adult retina.

Introduction
Ca^{2+} signaling is required for many fundamental aspects of neuronal function, such as signaling at pre- and postsynaptic sites, gene activation, growth, development and survival [1]. Ca^{2+}-binding proteins sense and transduce Ca^{2+} signals by undergoing conformational changes upon Ca^{2+} binding, and consequently regulating target proteins. The largest group of Ca^{2+}-binding proteins are structurally conserved EF-hand containing proteins belonging to the calmodulin superfamily. Within this superfamily, the vertebrate-specific CaBP subfamily comprises CaBP1, CaBP2, CaBP4 and CaBP5 in mammals [2] [3] [4]. In zebrafish, the CaBP subfamily is expanded to 8 members encoded by *cabp1a, cabp1b, cabp2a, cabp2b, cabp4a, cabp4b, cabp5a* and *cabp5b* [5], most likely due to duplicate gene retention after teleost-specific whole genome duplication [6].
CaBPs are specifically expressed in parts of the nervous system, including the retina [2] [5] [7]. The role of CaBP4 in retinal function is currently best understood. CaBP4, being within the retina specifically expressed in photoreceptors, has an established role in retinal disease and photoreceptor synaptic function [7] [8] [9] [10] [11] [12] [13] [14]. Functions of CaBPs expressed in second-order retinal neurons (i.e. bipolar and horizontal cells) are less well understood [15] [16]. Bipolar cells of the mouse retina have been reported to express CaBP5 [2], and *cabp2* transcripts have also been detected in this cell type [17]. Characterization of the CaBP family in zebrafish has revealed two orthologues for each mammalian CaBP-encoding gene, and expression analysis was performed in the developing embryo [18]. Similar to mammals zebrafish *cabp4b* is expressed in photoreceptors, while no specific expression pattern was detected for *cabp4a*. *cabp1a* and *cabp1b* are both expressed in amacrine cells. Like the mammalian orthologues, *cabp2a* and *cabp5b* are expressed in retinal bipolar cells during development, while *cabp2b* expression was reported exclusively in hair cells. *cabp5a* expression has not been detected at these stages [5].

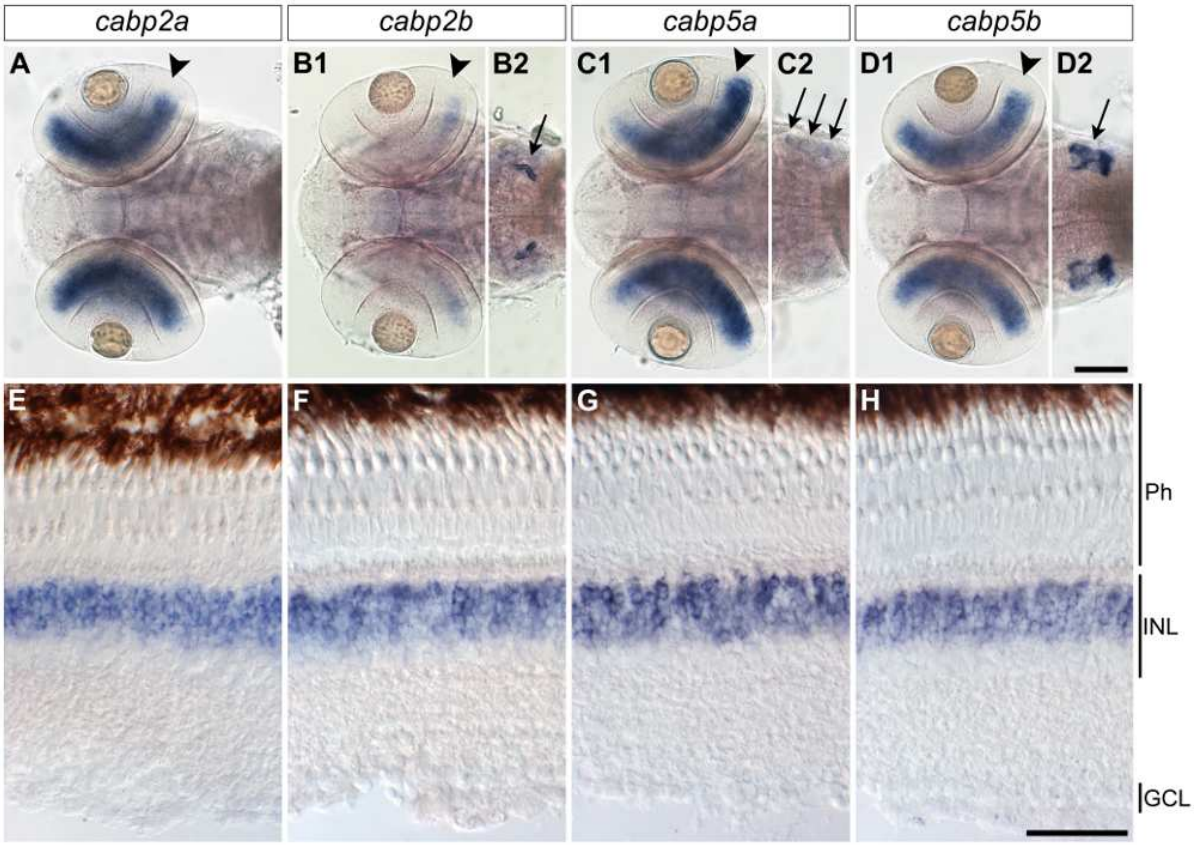

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Figure Legend

Expression patterns of *cabp2a, cabp2b, cabp5a* and *cabp5b* analyzed by RNA *in situ* hybridization. (A-D) Expression in 3 day-old zebrafish larvae. **(A, B1, C1, D1)** Within the retina, all four genes are expressed in the inner nuclear layer (INL, arrowheads). Note strong labeling of the INL by *cabp2a, cabp5a* and *cabp5b* in contrast to comparatively weak *cabp2b* signal. **(B2, C2, D2)** In addition to the retina, *cabp2b*, *cabp5a* and *cabp5b* are also expressed in the inner ear (arrows). **(E-H)** In the adult zebrafish retina, all four genes are exclusively expressed in the INL. While central and distal regions of the INL are labeled, the proximal region remains free from staining. Scale bars correspond 100 µm (D2, applies to A-D) and 50 µm (H, applies to E-H). GCL, ganglion cell layer, INL, inner nuclear layer; Ph, photoreceptor layer.

Results & Discussion

In order to study expression *of cabp2a, cabp2b, cabp5a* and *cabp5b* in the retina, we performed RNA *in situ* hybridization on developing whole-mount zebrafish and on adult retinal sections. In 3 day-old zebrafish larvae, all four genes studied show clear expression in the retinal inner nuclear layer (INL) (Fig.1 A-D). While staining for *cabp2a, cabp5a* and *cabp5b* results in strong labeling of the INL (Fig.1 A,B1,C1,D1), *cabp2b* staining is only weak in the retina (Fig.1 B1), also when compared to strong *cabp2b* staining observed in the inner ear (Fig.1 B2). Expression in the inner ear can also be detected for *cabp5a* and *cabp5b* (Fig.1 C2,D2). In the adult retina, all four *cabps* examined show exclusive and strong staining in the INL (Fig.1 G-H). Based on the shape and location of labeled cell bodies, all four genes are expressed in bipolar cells (central and distal INL), and possibly in horizontal cells (distal INL). The proximal INL, harboring amacrine and displaced ganglion cells, is devoid of staining.

Expression patterns of *cabp2a* and *cabp5b* in developing retina and ear are consistent with the findings by Di Donato *et al.* [5]. While expression of *cabp2b* has previously

been reported only in sensory hair cells, we detected weak expression in the retinal INL, likely reflecting low abundance of *cabp2b* transcripts in this region. Here, we show for the first time expression of *cabp5a* expression, which is strongly expressed in the retinal INL and in the inner ear. Our finding that expression of *cabp2a, cabp2b, cabp5a* and *cabp5b* is maintained up to adulthood strongly suggests functions in the mature retinal INL.

So far, no reports on zebrafish CaBP function exist. Studies of CaBP5 in mammalian cell culture and mouse retina suggest an important modulatory role in retinal signaling [16], possibly by regulation of voltage-gated Ca^{2+} channels [17] and a function in synaptic vesicle recruitment [18]. Since expression of *cabp5* orthologues is conserved between mammals and zebrafish, it is well conceivable that *cabp5a* and *cabp5b* have similar tasks.

The diversity of Ca^{2+}-binding proteins expressed in the nervous system allows finely-tuned Ca^{2+} signaling through their different Ca^{2+} affinities, subcellular and cellular localizations and different binding partners, making them non-redundant regulators of neuronal signaling [1]. This and other studies [2] [5] [15] suggest that bipolar cells exploit a repertoire of CaBPs for decoding Ca^{2+} signals, laying out the framework for further functional studies.

We demonstrate expression of four genes encoding CaBP subfamily members in the retinal INL of zebrafish*: cabp2a, cabp2b, cabp5a* and *cabp5b.* Since all four genes are expressed in the INL of both developing and adult zebrafish, our findings suggest functions of *cabp2a, cabp2b, cabp5a* and *cabp5b* in Ca^{2+} signaling in mature retinal neurons, besides possible developmental roles.

We show expression that strongly implies, but does not prove, function. The position of the cell bodies in the retina strongly suggests expression in bipolar and horizontal cells, but is not a formal proof.

The study implies a functional of these CaBPs in retinal processing. The obvious next step is to genetically manipulate these genes (e.g. by CRISPR/Cas9 genome editing) and assess altered retinal function in these fish. Due to overlapping expression patterns of paralogues in the INL, it might be necessary to generate double knockouts to observe a phenotype.

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201604000009.

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Ethics Statement

All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the local authorities (Veterinäramt Zürich TV4206).

Citations

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